Korea Research Institute of Chemical Technology

Analysis Results

Date received	September 30 2014	Client	CNL
Name of sample	Silver ion water		

Below are the results of efficacy evaluation of the compound provided by the client.

October 14 2014

Head of the Korea Research Institute of Chemical Technology

Report on the effectiveness of silver ion water on the prevention of plant diseases

■ Title of experiment: Effectiveness of silver ion water on the prevention of plant diseases at CNL's request.

■ Client: CNL

■ Test period: September 2014 – October 2014.

■ Tester: Choi Gyeongja
Environmentally-friendly new material research group at the Korea Research Institute of Chemical Technology

This is to report the results of the 'validation of in vivo disinfection activity of silver ion water' at CNL's request.

■ Preventive effects on seven subject diseases after different numbers of silver ion water treatment.

1. Test methods

Subject diseases: Plant diseases used for the experiment to validate in vivo disinfection activity of silver ion water are as follows:

Table 1. Plant diseases subject to the validation of preventive effects against plant diseases

Plant disease (Korean)	Plant disease (English)	Pathogen	Abbreviation
벼 도열병	Rice blast	*Magnaporthe oryzae*	RCB
벼 잎집무늬마름병	Rice sheath blight	*Rhizoctonia solani*	RSB
토마토 잿빛곰팡이병	Tomato gray mold	*Botrytis cinerea*	TGM
토마토 역병	Tomato late blight	*Phytophthora infestants*	TLB
밀 붉은녹병	Wheat leaf rust	*Puccinia recondite*	WLR
보리 흰가루병	Barley powdery mildew	*Blumeria graminis f. sp. hordei*	BPM
고추 탄저병	Pepper anthracnose	*Collectotrichum coccodes*	PAN

Preparation and treatment of drug: Tween 20 as surfactant was added to 10 ppm silver ion water produced by the client's silver ion water maker to finally reach a concentration of 250 ppm in the drug solution. Two pots of seedlings were used per treatment for each subject disease, cultivated in a greenhouse and the drug applied evenly by a hand sprayer.

Inoculation of pathogens and investigation of plant disease:
One day after the final drug treatment, each of the following pathogens was inoculated as follows:

For RCB, *Magnaporthe oryzae* KI-1113a strain was inoculated to a rice polish agar medium (rice polish 20g, dextrose 10g, agar 15g, and distilled water 1 l) and cultured for two weeks at 25℃. The surface of the cultured medium was scraped by a rubber polishman to remove submerged hyphae and spores were formed on a shelf with fluorescent lighting (25 - 28℃) for 48 hours. For the inoculation of RCB, a spore suspension at a certain concentration (10 x 10^6 conidia/ml) was made with conidiospores and sterilized distilled water and sprayed over Chucheong rice plant (with 2 – 3 foliage leaves). The inoculated rice plant was left in a dark wet room for 24 hours and in a constant temperature and humidity room at 26℃ with a relative humidity of at least 80% for five days. Then diseased rate (%) was investigated.

For RSB, a moderate amount of wheat bran was sterilized in a 1L culture bottle. *Rhizoctonia solani* AG-1 strain was inoculated to a sterilized medium and cultured for seven days at 25℃. The cultured mycelium lumps were finely ground and inoculated by pouring over the pot of Chucheong rice plants with 3 – 4 leaves, which were brought to disease outbreak by culturing in a wet room (25℃) for seven days. The outbreak of the disease was observed by investigating diseased rate of the leaf sheath.

For TGM, *Botrytis cinerea* was inoculated to a potato agar medium and cultured for seven days in a constant temperature device (dark conditions) at 25℃, followed by culture for seven days with 12 hours of light exposure per day to form spores. For the inoculation of TGM, the cultured spores were

harvested with a potato dextrose broth. The concentration of the spores was set to 5 x 10^5 conidia/ml with a hemocytometer and the spores were inoculated by spraying over tomato seedlings (with 2 – 3 foliage leaves). The inoculated tomato seedlings were brought to disease outbreak by leaving in a wet room (with a relative humidity over 95%) at 20℃ for three days. Diseased rate (%) was observed on the leaves.

For TLB, *Phytophthora infestants* PIT strain was inoculated to an oatmeal agar medium and cultured for seven days in a constant temperature device (dark conditions) at 20℃, followed by culture for seven days with 16 hours of light exposure per day to form zoosporangium. The zoosporangium were harvested by adding sterilized distilled water and the concentration of spores was investigated with a hemocytometer under an optical microscope to make a spore suspension of 5 x 10^5 sporangia/ml, which was cold-processed for 1 hour at 4℃ to bring out zoospores and sprayed over tomato seedlings (with 2 – 3 foliage leaves). The inoculated tomato seedlings were left in a wet room at 20℃ for two days and then moved to a constant temperature room at 20℃, and diseased rate (%) was investigated.

For WLR, given the pathogen *Puccinia recondite* is a parasite, it was subcultured directly on plants in the laboratory, and spores formed on wheat seedlings were used as the source of inoculation. To investigate to efficacy of the drug, five wheat seeds (‘Eunpa’) were planted per pot (diameter: 6.5 cm) and cultured for eight days in a greenhouse, and a spore suspension (spores 0.67g/L) was sprayed over wheat seedlings with one leaf. The inoculated wheat seedlings were left in a wet room at 20℃ for one day and moved to a constant temperature and humidity room at 20℃ with a relative temperature of 60% to bring to disease outbreak. Diseased rate was investigated seven days after inoculation.

For BPM, given the pathogen *Blumeria graminis f. sp. horder* is a parasite, it was subcultured directly on barley seedlings in the laboratory, and spores formed on the barley seedlings were used as the source of inoculation. To investigate to efficacy of the drug, five barley seeds (‘Dongbori’) were planted per pot (diameter: 6.5 cm) and cultured for eight days in a greenhouse, and BPM spores were dusted over barley seedlings with one leaf. The inoculated barley seedlings were left in a constant temperature and humidity room at 20℃ with a relative temperature of 60% for seven days to bring to disease outbreak, and diseased rate was investigated.

Calculation of control rate: Based on the diseased rates obtained from the experiments, control rate was calculated by the following formula:

Control rate (%) = (1 – diseased rate of treated group / diseased rate of untreated group) x 100.

2. Results and discussions

To investigate the effect of silver ion water on the prevention of plant diseases, a 10 ppm silver ion water solution was applied twice a day from Day 1 to Day 6. One day after the last drug treatment, pathogens of seven plant diseases were inoculated and brought to outbreak, and the diseases were investigated. In other words, this experiment was to prevent plant diseases by using the client's intended silver ion water as agricultural water. The results showed that the more times silver ion water treatments were conducted against RCB, TLB, WLR, and PAN, the stronger control effects were observed (Table 2). In particular, silver ion water showed a 96% control rate when treated twice for one day against RCB. Among the seven plant diseases, little control effects were observed for RSB, TGM, and BPM

From the results, it is thought that silver ion water could be used as a wide range disinfectant to control a number of plant diseases by repeatedly applying it using hydration methods such as a sprinkler.

Table 2. Ion water's preventive effects on seven plant diseases

Treatment	RCB[a]	RSB	TGM	TLB	WLR	BPM	PAN
2 times for 1 day (Thu)	96[b]	0	0	43	73	0	85
4 times for 2 days (Wed, Thu)	100	0	0	93	73	0	92
6 times for 3 days (Tue, Wed, Thu)	100	0	0	94	80	0	94
8 times for 4 days (Mon, Tue, Wed, Thu)	100	5	14	93	80	0	93
10 times for 5 days (Sun, Mon, Tue, Wed, Thu)	100	0	14	96	83	0	90
12 times for 6 days (Sat, Sun, Mon, Tue, Wed, Thu)	100	5	14	94	83	0	90

a RCB, Rice blast; RSB, Rice sheath blight; TGM, Tomato gray mold; TLB, Tomato late blight; WLR, Wheat leaf rust; BPM, Barley powdery mildew; and PAN, Pepper anthracnose.

b Control rate (%).

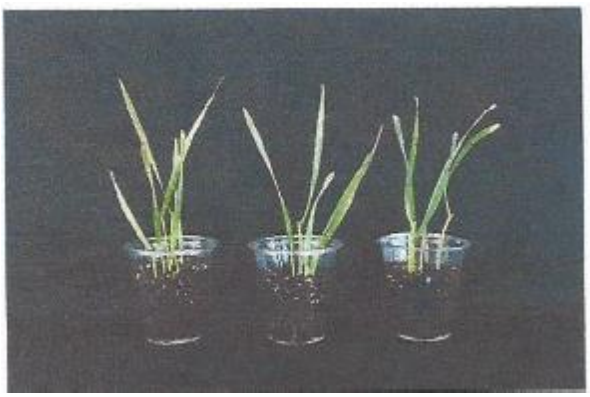
Table 3. Plant disease preventive effects of single treatment of control drugs one day prior to inoculation

Chemical	Con (μg, ml)	RCB[a]	RSB	TGM	TLB	WLR	BPM	PAN
Blasticidic-S	50	100[b]						
	1	50						
Tricyclazole	10	100						
	0.5	90						
Validamycin	50		100					
	5		90					
Flutolanil	50		100					
	20		100					
Fludioxonil	50			100				
	5			36				
Fenheximide	100			100				
	20			75				
Dimenthomorph	10				100			
	2				79			
Chlorothalonil	100				100			
	50				99			
Flusilazole	10					100		
	2					73		
Carboxin	50					100		
	20					43		
Flusilazole	10						100	
	0.5						80	
Benomyl	100						100	
	1						90	
Dithianon	50							55
	10							15

a RCB, Rice blast; RSB, Rice sheath blight; TGM, Tomato gray mold; TLB, Tomato late blight; WLR, Wheat leaf rust; BPM, Barley powdery mildew; and PAN, Pepper anthracnose.
b Control rate (%).

<RCB, non-treated, 1 day silver ion water treated, tricyclazole 10 ppm (from left)>

<TLB, non-treated, 2 day silver ion water treated, chlorothalonil 100 ppm (from left)>



<WLR, non-treated, 3 day silver ion water treated, flusilazole 10 ppm (from left)>

<PAN, non-treated, 1 day silver ion water treated, dithianon 50 ppm (from left)>